

In the Title:

Please amend the title as follows:

Novel Method for Gene Cloning Compositions for Isolating a cDNA Encoding a
Membrane-Bound Protein

Amendments to the Specification:

Please insert the following paragraph on page 1 of the specification, after the title:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of and claims priority to U.S. Application Serial No. 09/700,820, filed on November 20, 2000, which claims priority to International Application PCT/JP99/02341, filed April 30, 1999, which claims priority to Japanese Application No. 10/279876, filed October 1, 1998 and Japanese Application No. 10/138652, filed May 20, 1998. The entire contents of all of these applications are herein incorporated by reference.

Please replace the paragraph beginning at page 15, line 33, with the following amended paragraph:

First, in order to amplify the app. 1.1 kb fragment containing the cDNA encoding SR345 from the cDNA of IL-6 receptor (Yamasaki, K. et al., Science (1988) 241, 825-828), PCR primers IL6R1 (SEQ ID NO: 3) and IL6R2 (SEQ ID NO: 4) were designed. A PCR reaction mixture (100 μ l) containing 10 mM Tris-HCl (pH8.3), 50 mM KCl, 0.1 mM dNTPs, 1.5 mM MgCl₂, 100 pmol each of the above-mentioned primers, 100 ng of template DNA (cDNA encoding IL-6 receptor), and 5 units of AmpliTaq Gold enzyme was subjected to denaturation at 94°C, incubated 30 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, and finally, incubated for 10 min at 72°C. The amplified DNA fragment was collected and purified by 1% low melting point agarose gel electrophoresis, digested by EcoRI, and inserted into the EcoRI site of expression vector pCOS1. This was transfected into *E. coli*, and plasmids were prepared to obtain those in which the DNA fragment was inserted in the right direction.

The expression vector pCOS1 was constructed from plasmid HEF-PMh-g γ 1 (see WO92/19759) by deleting contained genes by EcoRI and SmaI digestion, and ligating with EcoRI-NotI-BamHI Adaptor (TaKaRa).

Please replace the paragraph beginning at page 17, line 18, with the following amended paragraph:

A PCR reaction mixture (100 μ l) containing 10 mM Tris-HCl (pH8.3), 50 mM KCl, 0.1 mM dNTPs, 1.5 mM MgCl₂, 100 pmol each of the above-mentioned primers, 100 ng of template DNA, and 5 units of AmpliTaq Gold enzyme was subjected to denaturation at 94°C for 9 min, incubated 30 cycles of 30 sec at 94°C and 1 min at 60°C, and finally, incubated for 5 min at 60°C. The PCR product was purified using at 1.5% low melting point agarose gel.

Please replace the paragraph beginning at page 18, line 6, with the following amended paragraph:

The DNA fragment encoding H chain and L chain V regions prepared above, and the DNA fragment encoding the linker region were assembled by the PCR method, and backward primer TMT1 and forward primer TMT2 (SEQ ID NO: 13) were added to amplify the full-length DNA fragment encoding scFv of humanized PM1. The forward primer TMT2 was designed in such a manner that it should hybridize to the DNA sequence encoding HindIII restriction enzyme recognition site and FLAG peptide, and also comprise two repetitive translation stop codons, and the EcoRI restriction enzyme recognition site. The primary PCR was conducted as follows: 98 μ l of a PCR reaction mixture containing 10 mM Tris-HCl (pH8.3), 50 mM KCl, 0.1 mM dNTPs, 1.5 mM MgCl₂, app. 100 ng each of the above PCR products, and 5 units of AmpliTaq Gold enzyme was subjected to denaturation first at 94°C, and then 2 cycles of 2 min at 94°C, 2 min at 55°C, and 2 min at 72°C were done to ligate each DNA fragment. The secondary PCR was done in the following manner: 100 pmol of each primer was added to the above PCR reaction solution, 30 cycles of 30 sec at 94°C and 1 min at 60°C were done, and finally, the mixture was incubated for 5 min at 60°C.

Please replace the paragraph beginning at page 33, line 22, with the following amended paragraph:

Next, the gene encoding humanized PM1 antibody single-chain Fv was amplified in the same manner. PCR was conducted under the same condition as mentioned above using EF1 (SEQ ID NO: 28) as the backward primer, SCP-Mu (SEQ ID NO: 37) as the forward primer, and pTMT-scFv as template DNA. The forward primer SCP-Mu was designed in such a manner that it should hybridize to the nucleotide sequence encoding single-chain Fv C terminus shown in SEQ ID NO:[A]23, and comprise a nucleotide sequence that was complementary to the 5' end of the μ chain partial sequence gene amplified by PCR. The PCR product was purified in the same manner.

Please replace the Sequence Listing with the Sequence Listing filed December 20, 2001 in the parent application, U.S. Application 09/700,820. A paper copy of the Sequence Listing filed December 20, 2001 is provided herewith. Applicant also encloses a Request Under 37 CFR 1.82(e) to Transfer Computer Readable Form.

Please delete the previous abstract at page 42 and add the following new abstract:

A method and kit are provided for selectively isolating genes encoding membrane-bound proteins by fusing proteins to a secretable protein having a binding affinity to an antigen (e.g., an antibody), expressing these fusions in host cells, and selectively isolating individual host cells by their ability to bind the antigen via the fusion protein. Host cells expressing fusion proteins that do not contain a membrane binding domain will be relatively unable to bind the antigen, since the fusion proteins in those cells are secreted and unattached to their host cells. The method, vector, and kit disclosed herein are particularly useful for identifying genes for membrane-bound proteins represented in cDNA libraries.